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EP 0 335 541 B1

Description

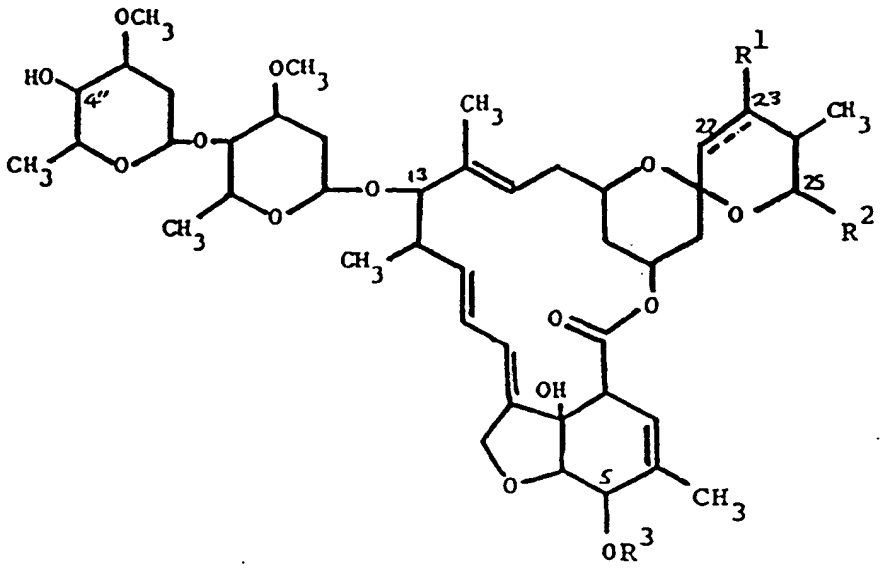
This invention relates to antiparasitic agents and in particular to compounds related to the avermectins but having a novel substituent group at the 25-position and to processes for their preparation and compositions thereof.

The avermectins are a group of broad spectrum antiparasitic agents referred to previously as the C-076 compounds. They are produced by fermenting a strain of the microorganism Streptomyces avermitilis under aerobic conditions in an aqueous nutrient medium containing inorganic salts and assimilable sources of carbon and nitrogen. The isolation and the chemical structure of the eight individual components which make up the C-076 complex is described in detail in British Patent Specification no. 1573955.

In our European Patent Applications publication nos. 0214731 and 0284176 and in British Patent Application no. 8726730 we describe the preparation of compounds related to the avermectins but having an unnatural substituent group at the 25-position in place of the isopropyl or sec-butyl group which is present in the naturally occuring avermectins.

The present invention provides a further series of semi-synthetically derived novel compounds wherein the 25-position substituent is an alkenyl or substituted alkenyl group. The compounds possess a broad spectrum of activity against insect pests, acari, free-living nematodes and parasites affecting humans and animals.

Thus, according to the present invention there are provided compounds having the formula:-



(I)

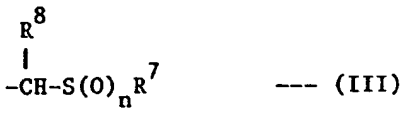
wherein the broken line at the 22-23 position represents an optional double bond and wherein either R^1 is H and the double bond is present or is OH and the double bond is absent;

R^2 is a group of the formula: -CH=CH-R^6; and

R^3 is H or CH_3;

wherein R^6 is H or phenyl or substituted phenyl wherein said substituent is fluorine, chlorine, C_1-C_4 alkyl, C_1-C_4 alkoxy, C_1-C_4 alkylthio, hydroxy(C_1-C_4)alkyl, cyano, aminosulphonyl, C_2-C_6 alkanoyl, C_2-C_6 alkoxycarbonyl, nitro, trifluoromethyl, trifluoromethoxy, amino or mono or di-C_1-C_4 alkylamino.

The invention also includes compounds of the formula (I) above wherein R^1 and R^3 are as previously defined and R^2 is a group of the formula:-



--- (III)

wherein R^7 is C_1-C_4 alkyl, R^8 is methyl and n is 1 or 2. These compounds are synthetic intermediates for the compounds of formula (I) as well as being active antiparasitic agents in their own right.

In the above definition, alkyl groups containing 3 or more carbon atoms may be straight or branched chain.

Preferred compounds include those derivatives wherein R^2 is -CH=CH_2 or -CH=CH-R^6 wherein R^6 is 4-trifluoromethoxyphenyl; the avermectin B1 derivatives wherein R^3 is hydrogen, R^1 is hydrogen and the 22,23 double bond is present being especially preferred.

The C-076 complex comprises eight distinct but closely related compounds described as C-076 A1a, A1b, A2a, A2b, b1a, b1b, B2a and B2b. The "a" series of compounds refers to the natural avermectins wherein the 25-substituent is (S)-sec-butyl and the "b" series to those wherein the 25-substituent is isopropyl. The designations "A" and "B" refer to avermectins wherein the 5-substituent is methoxy or hydroxy, respectively, and the numeral "1" refers to avermectins wherein a double bond is present at the 22-23 position, and numeral "2" to avermectins lacking the 22-23 double bond and having a hydrogen at the 22-position and hydroxy at the 23 position.

In this specification, the "a" and "b" identifiers have been dropped, however, identifiers A1, A2, B1 and b2 have been retained to refer to non-natural avermectins having the structural features corresponding to those of the natural avermectins as noted above.

The compounds of formula (I) are prepared by a number of different processes according to the invention:-
a) Compounds of the formula (I) wherein R^2 is -CH=CH_2 are prepared from the corresponding C-25 alkylthioalkyl compound of formula (I) wherein R^2 is:

--- (II)

wherein R^7 is C_1-C_4 alkyl, by a process which involves, first oxidation to give the corresponding sulphoxide or sulphone wherein R^2 is:

--- (III)

wherein n is 1 or 2, followed in the case of the sulphoxides by a thermal elimination or, in the case of the sulphones, by a base catalysed elimination reaction.

The oxidation step is achieved in a conventional manner by treating the alkyl-sulphide, in solution, with an oxidising agent. A variety of oxidants may be used for this step but for best results for preparation of the sulphoxides, a reagent is used which avoids over-oxidation to the sulphone. Thus preferred oxidants would be for example meta-chloroperbenzoic acid tertiary-butyl hypochlorite or sodium metaperiodate, meta-chloroperbenzoic acid being the reagent of choice. The reaction is generally achieved by adding one equivalent of the oxidant to cooled solution of the sulphide in an inert organic solvent, for example dichloromethane. The course of the reaction can be followed by thin layer chromatography and the reaction is generally substantially complete after several hours. Excess oxidant is destroyed, for example by the addition of dimethylsulphide, and the product is then isolated in a conventional manner and further purified, if desired, by chromatography. The sulphones can be prepared following a similar procedure but using excess oxidising agent for a longer period of time.

The elimination step to give the alkene is generally achieved by heating the sulphoxide in a high-boiling organic solvent, for example by heating in 1,2,4-trichlorobenzene at 175°C for a period of one or two hours. Again the product is isolated in a conventional manner, typically by adsorption onto a silica column, followed by elution with an appropriate solvent. Further purification can be achieved, if desired, by column chromatography or high pressure liquid chromatography.

A preferred R^2 group for use in the elimination reaction is the 1-methylsulphinylethyl which yields compounds of formula (I) wherein R^2 is ethenyl.

The intermediate sulphoxides and sulphones of formula (I) wherein R^2 is as defined in formula (III), in addition to being useful synthetic intermediates are also active anti-parasitic agents in their own right and form a further aspect of this invention.

The starting C-25 alkylthioalkyl avermectin derivatives of formula (I) wherein R^2 is as defined by formula (II) are prepared by adding the appropriate alkylthioalkyl carboxylic acid to a fermentation of an avermectin producing organism as described in EP-A-0214731 or our European patent application no. 88300353.5 or British patent application no. 8726730.

(b) Compounds of the formula (I) wherein R^2 is -CH=CHR^6 and R^6 is substituted or unsubstituted phenyl, are prepared from the corresponding compounds of formula (I) where R^2 is:

$$-CH = CH_2 \quad (IV)$$

by a palladium catalysed reaction with a compound of the formula R^9-L wherein R^9 is substituted or unsubstituted phenyl and L is a suitable leaving group, e.g. bromine, iodine or organomercury. Appropriate reagents and conditions for this step (the Heck reaction), are described for example in Organic Reactions published by John Wiley & Sons, Volume 27 (1982). Typically the compound of formula (I) wherein R^2 is as defined by formula (IV) above, in an organic solvent e.g. acetonitrile, is warmed with an excess of an aryl halide, generally the iodide, in the presence of palladium acetate and a tertiary amine. The reaction is generally complete after 24 hours at a temperature of 50-60°C and the product is then isolated and purified by conventional techniques.

The starting materials of formula (I) wherein R^2 is as defined by formula (IV) above are obtained from the corresponding C-25 alkylthioalkyl compound as described in process (a) above.

As previously mentioned the compounds of the invention are highly active antiparasitic agents having particular utility as anthelmintics, ectoparasiticides, insecticides, acaricides and animal growth promotants.

Thus the compounds are effective in treating a variety of conditions caused by endoparasites including, in particular, helminthiasis which is most frequently caused by a group of parasitic worms described as nematodes and which can cause severe economic losses in swine, sheep, horses and cattle as well as affecting domestic animals and poultry. The compounds are also effective against other nematodes which affect various species of animals including, for example, Dirofilaria in dogs and various parasites which can infect humans including gastro-intestinal parasites such as Ancylostoma, Necator, Ascaris, Strongyloides, Trichinella, Capillaria, Trichuris, Enterobius and parasites which are found in the blood or other tissues and organs such as filiarial worms and the extra intestinal stages of Strongyloides and Trichinella.

The compounds are also of value in treating ectoparasite infections including in particular arthropod ectoparasites of animals and birds such as ticks, mites, lice, fleas, blowfly, biting insects and migrating dipterous larvae which can affect cattle and horses.

The compounds are also insecticides active against household pests such as the cockroach, clothes moth, carpet beetle and the housefly as well as being useful against insect pests of stored grain and of agricultural plants such as spider mites, aphids, caterpillars, fire ants, termites and against migratory orthopterans such as locusts.

The compounds of formula (I) are administered as a formulation appropriate to the specific use envisaged and to the particular species of host animal being treated and the parasite or insect involved. For use as an anthelmintic the compounds are preferably administered by injection, either subcutaneously or intramuscularly, alternatively they may be administered orally in the form of a capsule, bolus, tablet or liquid drench, or they may be administered as a pour-on formulation or as an implant. Such formulations are prepared in a conventional manner in accordance with standard veterinary practice. Thus injectable formulations may be prepared in the form of a sterile solution or emulsion. Capsules, boluses or tablets may be prepared by mixing the active ingredient with a suitable finely divided diluent or carrier, additionally containing a disintigrating agent and/or binder such as starch, lactose, talc, or magnesium stearate. A drench formulation may be prepared by dispersing the active ingredient in an aqueous solution together with dispersing or wetting agents. These formulations will vary with regard to the weight of active compound depending on the species of host animal to be treated, the severity and type of infection and the body weight of the host. Generally for oral or parenteral administration, a dose of from about 0.001 to 10 mg per kg, preferably 0.01 to 1 mg/kg of animal body weight given as a single dose or in divided doses for a period of from 1 to 5 days will be satisfactory but of course there can be instances where higher or lower dosage ranges are indicated and such are within the scope of this invention.

As an alternative the compounds may be administered with the animal feedstuff and for this purpose a concentrated feed additive or premix may be prepared for mixing with the normal animal feed.

For use as an insecticide and for treating agricultural pests, the compounds are ,applied as sprays, dusts, emulsions pour-on formulations and the like in accordance with standard agricultural practice.

For use as a growth promotant or for improving the lean meat to fat ratio in farm or domestic animals, the compounds may be administered with the animal feedstuff or drinking water. Alternatively they may be administered orally in the form of a capsule, bolus, tablet or liquid drench, or parenterally by injection or as an implant.

Such formulations are prepared in a conventional manner in accordance with standard veterinary practice.

For human use the compounds are administered as a pharmaceutically acceptable formulation in accordance with normal medical practice.

The invention is illustrated by the following Examples in which Examples 1-4 describe the preparation of compounds of formula (I) wherein R^2 is as defined in formula (III) and Examples 5-29 describe preparation of compounds of formula (I).

Fast atom bombardment (FAB) mass spectrometry was performed on a VG Model 7070E mass spectrometer using a sample matrix of glycerol, thioglycerol, water and sodium chloride. Electron impact (EI) mass spectrometry was performed using a VG model 7070E mass spectrometer. m/z values are quoted for the principal fragments. ^{1}H Nuclear magnetic resonance (NMR) spectral data were obtained on a Nicolet QE 300 spectrometer with a sample concentration of 5 mg/ml in deuteriochloroform. The chemical shifts are given in parts per million relative to tetramethylsilane.

EXAMPLE 1

25-(1-Methylsulphinylethyl)-avermectin B1 (formula I; R^1 = H, 22,23-double bond present, R^2 = -$CH(CH_3)SOCH_3$, R3 = H).

A solution of meta-chloroperbenzoic acid (0.44 g, 85%) in dichloromethane (7 ml) was added dropwise to a stirred, cooled solution of 25-(1-methylthioethyl)-avermectin B1 (1.64 g) in dichloromethane (40 ml) at -70°C. After 5 hours, t.l.c. showed no starting material remaining. Several drops of dimethylsulphide were added and the mixture was allowed to warm to room temperature. The solution was then extracted with saturated aqueous sodium bicarbonate solution, and the organic phase dried over sodium sulphate and evaporated to yield the product as an oil (1.57 g, 95%) which was generally used for the next stage without further purification. Purification, when required, was carried out by adsorbing the product, in dichloromethane, onto a Waters silica Sep-Pak (trade mark) column, washing with ethyl acetate and then eluting the product with chloroform containing 5% methanol. The solution was evaporated and the residue was re-dissolved in aqueous methanol. Evaporation gave the required product as a white solid containing a mixture of epimeric diastereomers which could be further resolved by reverse-phase high-pressure liquid chromatography if required.

FAB mass spectrometry: (M + Na^+) observed at m/z 929 (theoretical 929).

EI mass spectrometry: 261, 256, 242, 236, 227, 145, 113 and 87.

^{1}H NMR as expected for a B1 avermectin with characteristic peaks for the C-25 side-chain at 2.62 (3H, s, $SOCH_3$), 1.55 (3H, d, $CH(\underline{CH_3})SOCH_3$).

EXAMPLE 2

25-(1-Methylsulphinylethyl)-avermectin A2 (formula I; R^1 = OH, double bond absent, R^2 = -$CH(CH_3)SOCH_3$, R^3 = CH_3).

This was prepared as described in Example 1, using meta-chloroperbenzoic acid (0.055 g, 85%) and 25-(1-methylthioethyl)-avermectin A2 (0.176 g) to yield 184 mg of the title product as a white powder after evaporation from aqueous methanol. The product is a mixture of epimeric diastereomers which can be further resolved by reverse-phase liquid chromatography if required.

FAB mass spectrometry: (M + Na^+) observed at m/z 961 (theoretical 961).

EI mass spectrometry: 588, 536, 511, 339, 275, 145, 113 and 87.

^{1}H NMR as expected for an A2 avermectin with characteristic peaks for the C-25 side-chain at 2.65 (3H, s, $SOCH_3$), 1.55 (3H, d, $CH(\underline{CH_3})SOCH_3$).

EXAMPLE 3

25-(1-Methylsulphonylethyl)-avermectin A2 (formula I; R^1 = OH, double bond absent, R2 = -$CH(CH_3)SO_2CH_3$, R3 = CH_3).

A solution of meta-chloroperbenzoic acid (0.006 g, 85%) in dichloromethane (0.3 ml) was added dropwise to a stirred, cooled solution of 25-(1-methylthioethyl)-avermectin A2 (0.015 g) in dichloromethane (4 ml) at -70°C. The reaction mixture was allowed to warm to -18°C and stirred at that temperature overnight. Several drops of dimethylsulphide were added and the mixture was allowed to warm to room temperature. The solution was then extracted with saturated aqueous sodium bicarbonate solution, and the organic phase dried over

sodium sulphate and evaporated to yield the product as an oil (13 mg). The crude product was purified by reverse-phase high-pressure liquid chromatography on a Beckman Ultrasphere ODS (trade mark) C18 column eluting with 30% aqueous methanol. Evaporation of the appropirate fractions gave the product as a white solid (9 mg).

FAB mass spectrometry: (M + Na^+) observed at m/z 977 (theoretical 977).

EI mass spectrometry: 648, 373, 355, 337, 289, 261, 243, 145, 113, 87.

^1H NMR as expected for a A2 avermectin with characteristic peaks for the C-25 side-chain at 3.0 (3H, s, $SO_2\underline{CH_3}$), 1.55 (3H, d, $CH(\underline{CH_3})SO_2CH_3$).

EXAMPLE 4

25-Ethenyl-avermectin B1 (formula I; R^1 = H, 22,23-double bond present, R^2 = CH_2=CH-, R^3 = H).

A stirred solution of 25-(1-methylsulphinylethyl)- avermectin B1 (0.077 g) in 1,2,4-trichlorobenzene (3 ml) containing reprecipitated calcium carbonate (140 mg) was heated at 175°C under nitrogen for 1 hour. The mixture was cooled, diluted with dichloromethane and filtered. The filtrate was passed through a short silica column. The column was washed with dichloromethane and then the product was eluted using ethyl acetate. Evaporation of the ethyl acetate gave the required product as an oil (66 mg). The crude product (90% pure) was purified by reverse-phase high-pressure liquid chromatography on a Dupont Zorbax (trade mark) ODS C18 column eluting with a 23:77 mixture of water:methanol. Evaporation of the appropriate product containing fractions of the eluant gave the product as a white powder.

FAB mass spectrometry: (M + Na^+) observed at m/z 865 (theoretical 865).

EI mass spectrometry: 536, 275, 191, 163, 145, 139, 113, 95 and 87.

^1H NMR as expected for a B1 avermectin with characteristic peats for the C-25 side-chain at 5.85 (1H, m, $C\underline{H}$=CH_2), 5.3 (2H, m, CH=$C\underline{H_2}$).

EXAMPLE 5

25-Ethenyl-avermectin A2 (formula I, R^1 = OH, double bond absent, R^2 = -CH=CH_2, R^3 = CH_3).

This was prepared as described in Example 4 but starting with 25-(1-methylsulphinylethyl)-avermectin A2 (0.68 g) and heating in 1,2,4-trichlorobenzene (27 ml) containing reprecipitated calcium carbonate to yield the required product as an oil (490 mg after work-up). The crude product (90% pure) was purified by reverse-phase liquid chromatography on a Dynamax (trade mark) 60-A C18 column eluting with a 23:77 mixture of water:methanol. Evaporation of the appropriate product containing fractions of the eluant gave the product as a white powder.

FAB mass spectrometry: (M + Na^+) observed at m/z 897 (theoretical 897).

EI mass spectrometry: 568, 293, 275, 209, 179, 163, 145, 127, 113, 95 and 87.

^1H NMR as expected for a A2 avermectin with characteristic peaks for the C-25 side-chain at 5.8 (1H, m, $C\underline{H}$=CH_2), 5.3 (2H, m, CH=$C\underline{H_2}$).

EXAMPLES 6-18

25-(2-Phenylethenyl)-avermectin A2 (formula I, R^1 = OH, double bond absent, R^2 = -CH=CHC_6H_5, R^3 = CH_3).

A mixture of palladium acetate (10-50 mg) and a solution of 25-ethenyl-avermectin A2 (50 mg), iodobenzene (250 mg) and triethylamine (250 mg) in acetonitrile was stirred at 60°C for 24 hours. The reaction mixture was filtered and the filtrate was evaporated to dryness. The residue was taken up in methanol and the solution filtered and evaporated. The product, in dichloromethane, was adsorbed onto a Waters silica Sep-Pak (trade mark) column, washed with dichloromethane and the crude product was eluted with ethyl acetate. Purification by reverse-phase high pressure liquid chromatography on a 1 inch Dupont Zorbax (trade mark) ODS C18 column eluting with mixtures of methanol and water gave the product as a white solid (24 mg).

A range of similar compounds were prepared by the same method and on the same scale using the appropriate aryl iodide. Data are provided in Table 1.

TABLE 1

Example	C-25 Substituent	Class	Yield	^{1}H NMR signals for C-25 side-chain	FAB MS Ion observed/theor.	EI MS Fragmentation pattern
6	2-Phenylethenyl	A2	24 mg	7.4 (m,5H), 6.7 (d,1H), 6.25 (dd,1H)	973/973	369, 351, 337, 303, 215, 145, 129, 113, 95, 87
7	2-Phenylethenyl	B1	22 mg	7.4 (m,5H), 6.7 (d,1H), 6.3 (dd,1H)	941/941	351, 267, 218, 197, 169, 145, 127, 113, 95, 87
8	2-(4-Fluorophenyl) -ethenyl	A2	18 mg	7.4 (m,2H), 7.04 (m,2H), 6.66 (d,1H), 6.16 (dd,1H)	991/991	662, 369, 303, 145 127, 113, 95, 87
9	2-(4-Methylthio-phenyl)-ethenyl	A2	17 mg	7.35 (m, 2H), 7.22 (m, 2H) 6.64 (d, 1H) 6.2 (dd, 1H), 2.52 (s,3H)	1019/1019	415,190,178,145,113 87
10	2-(4-Methoxyphenyl) -ethenyl	A2	30 mg	7.38 (d,2H), 6.88 (d,2H), 6.63 (d,1H), 6.1 (dd,1H), 3.75 (s,3H)	1003/1003	381, 315, 175, 161, 145, 113, 95, 87

Example	C-25 Substituent	Class	Yield	^{1}H NMR signals for C-25 side-chain	FAB MS Ion observed/theor.	EI MS Fragmentation pattern
11	2-(4-hydroxymethyl-phenyl)-ethenyl	A2	14 mg	7.45 (d,2H), 7.36 (d,2H), 6.7 (d,1H), 6.25 (dd,1H), 4.7 (s,2H)	1003/1003	362,312,259,209, 145,87
12	2-(4-aminosulphonyl-phenyl)-ethenyl	A2	22 mg	7.8 (d,2H), 7.58 d,2H), 6.77 (d,1H), 6.42 (dd,1H)	1052/1052	351,290,242,225,145, 113,87
13	2-(4-acetylphenyl) -ethenyl	A2	25 mg	7.95 (d, 2H), 7.55 (d, 2H), 6.75 (d, 1H), 6.41 (dd, 1H), 2.62 (s, 3H)	1015/1015	393, 309, 187, 171, 145, 127, 113, 95, 87
14	2-(4-nitrophenyl) -ethenyl	A2	25 mg	8.22 (d,2H), 7.58 (d,2H) 6.8 (d,1H), 6.5 (dd,1H),	1018/1018	396, 312, 275, 257, 145, 127, 113, 95, 87
15	2-(4-trifluoro-methoxyphenyl)-ethenyl	A2	17 mg	7.5 (d,2H), 7.2 (d,2H), 6.7 (d,1H), 6.25 (dd,1H),	1057/1057	435, 369, 351, 323, 257, 145, 127, 113, 95, 87

Example	C-25 Substituent	Class	Yield	^{1}H NMR signals for C-25 side-chain	FAB MS Ion observed/theor.	EI MS Fragmentation pattern
16	2-(4-trifluoro-methoxyphenyl)-ethenyl	B1	19 mg	7.48 (d,2H), 7.2 (d,2H), 6.7 (d,1H), 6.28 (dd,1H),	1025/1025	696,281,215,145,113, 87
17	2-(4-methoxy-carbonylphenyl)-ethenyl	A2	19 mg	8.01 (d, 2H), 7.44 (d, 2H), 6.75 (d, 1H), 6.4 (dd, 1H), 3.92 (s, 3H)	1031/1031	702, 409, 377, 343, 325, 203, 171, 145, 127, 113, 95, 87
18	2-(4-formyl-phenyl)-ethenyl(1)	A2	9 mg	10.02 (s,1H), 7.88 (d,2H), 7.5 (d,2H), 6.78 (d,1H), 6.45 (dd,1H)	1001/1001	672, 379, 225, 173, 145, 127, 113, 95, 87

(1) By-product formed during the synthesis of Compound 14.

(2) Prepared at room temperature without triethylamine using methoxycarbonylmercuric acetate in place of the aryl iodide.

Preparation 1

25-(1-Methylthioethyl)avermectins A2 and B1

A frozen inoculum (2 ml) of a culture of Streptomyces avermitilis mutant organism ATCC 53568 was inoculated into 100 mls of a medium containing starch (2 g), Pharmamedia (Trademark) 1.5 g, ardamine pH (0.5 g) and calcium carbonate, and incubated in two 300 ml Erlenmeyer flasks at 28°C on a rotary shaker with a 2.5 cm throw at 170 r.p.m. for 2 days. The resultant vegetative growth was used to inoculate at a rate of 5% two Fernbach flasks containing 1 litre each of the seed medium for preparation of the second seed culture. These flasks were incubated under the same conditions, and after two days the total contents transferred to a 100 litre vessel containing 70 litres of the same medium and incubated at 28°C, for 2 days, with agitation at 350 r.p.m. and aeration at 70 litres/min. This third stage seed culture was then inoculated into a 2000 litre fermenter containing 1200 litres of a medium consisting of starch (100 kg), dipotassium hydrogen phosphate (1.2 kg), ferrous sulphate (120 g), calcium carbonate (8.4 kg) glutamic acid (0.72 kg), and manganous sulphate (120 g) at pH 7.0. Methylthiolactic acid (480 g) was added after 96 hours and again after 168 hours (240 g) and 216 hours (126 g). After 288 hours the mycelium was removed by filtration and extracted with acetone (2 x 410 litres). The acetone extract was concentrated to approximately 200 litres and extracted with ethyl acetate (3 x 205 litres). The combined ethyl acetate layers were concentrated to 10 litres and 10 litres methanol and 0.5 litres water were added. This solution was extracted with 20 litres hexane and the hexane layer separated and back-washed with 10 litres methanol and 0.5 litres water. The combined aqueous methanol layers were evaporated to dryness to give a dark brown oil (362 g). This oil was dissolved in dichloromethane (1.2 litres) and stirred for 1 hour with silica gel (300 g) and charcoal (150 g). The suspension was filtered and the filtrate evaporated to give a brown oil (275 g). A solution of this oil in isopropyl ether (350 ml) was dripped into stirred hexane (5 litres) at 10°C. After allowing the suspension to stand at 10°C overnight the precipitated light brown powder was recovered by filtration. The crude product (100 g), following precipitation from hexane, was chromatographed on silica gel (1 kg). The column was washed with diethyl ether/hexane (1:1) and the product then eluted with diethyl ether (7 litres) followed by ethyl acetate/diethylether (1:2, 3.5 litres) and ethyl acetate (3.75 litres). Fractions (250 ml) were collected. Fractions 19 to 31 were combined and evaporated to give a solid which consisted of a mixture of 25-(1-methylthioethyl)avermectin A2 and 25-(1-methylthioethyl)avermectin B1, (13 g). Fractions 32 to 39 were combined and evaporated to give a solid which contained 25-(1-methylthioethyl)avermectin B1, (3.49).

The later product (1.5 g) was further purified by high pressure liquid chromatography, on a C-18 Dynamax (trade mark Rainin) column (41.4 mm x 25 cm) eluting with a gradient of methanol and water from (75:25) to (80:20) over 104 minutes at a flow rate of 45 ml/min. Appropriate fractions were combined and evaporated to yield 360 mg of 25-(1-methylthioethyl)avermectin B1.

FAB mass spectrometry: (M^+ + Na) observed at m/z 913 (theoretical 913).

EI mass spectrometry: 584, 323, 261, 257, 233, 205, 145, 127, 113, 95 and 87.

^{1}H NMR ($CDCl_3$) 2.2 (3H, s, CH_3-S) 1.13 (3H, d, CH_3SCHCH_3).

Fractions 19 to 31 from the silica gel chromatography were further purified by chromatography on a C-18 Micro-Bondapack (trade mark) column (50 mm x 50 cm) in a water Prep 500 high pressure liquid chromatograph eluting with a mixture of methanol and water (77:23) at 50 ml/min. followed by chromatography of the A2 enriched fractions on a C-18 Dynamax (trade mark Rainin) column (41.4 mm x 25 cm) eluting with a gradient of methanol and water from 28:72 to 20:80 over 138 minutes at 45 ml/min. Appropriate fractions were combined to give 25-(1-methylthioethyl)avermectin A2 (320 mg).

FAB mass spectrometry: (M^+ + Na) observed at m/z 945 (theoretical 945).

EI mass spectrometry: 341, 323, 275, 263, 257, 239, 211, 187, 179, 145, 113, 111, 95 and 87.

^{1}H NMR ($CDCl_3$) 2.18 (3H,s,CH_3-S), 1.13 (3H,d,CH_3SCHCH_3).

TEST PROCEDURE

Anthelmintic Activity

Anthelmintic activity was evaluated against Caenorhabditis elegans using the in vitro screening test described by K. G. Simpkin and G. L. Coles in Parasitology, 1979, 79, 19, with a well concentration of 1 microgram per ml.

Insecticidal Activity

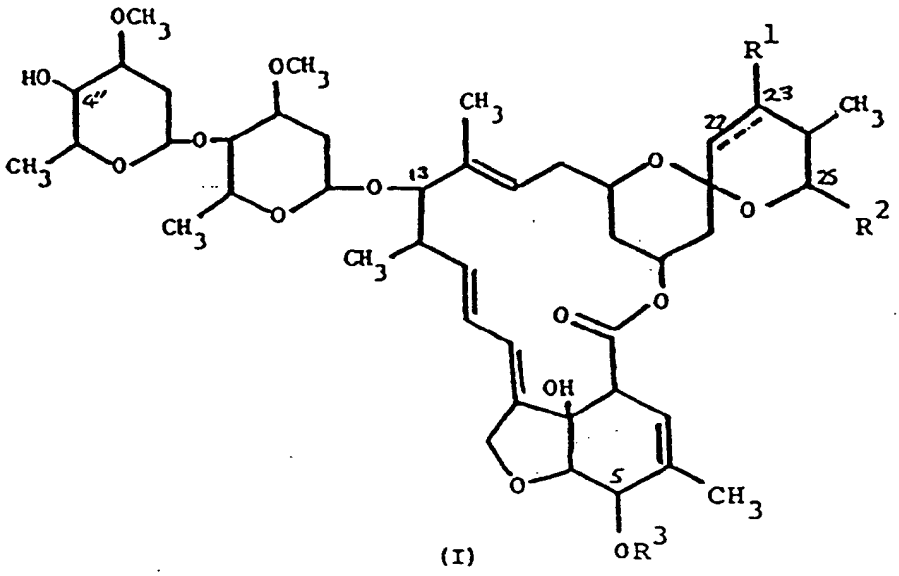
Activity against the larval stage of the blowfly Lucilia cuprina (Q strain) is demonstrated using a standard procedure in which first instar larvae are kept in contact with filter paper treated with test compound. The test compound is first applied to the paper as an acetone solution to give a concentration of the test compound of 1 milligram per square metre. The treated filter papers are then placed into tubes containing 1 ml of newborn calf serum and the first instars are added. The tubes are examined after 24 hours and the % of larvae killed recorded.

The compounds of the invention are active in the above tests, for most compounds, 100% of the worms or larvae were killed at the concentration of test compound stated.

Claims

Claims for the following Contracting States: AT, BE, CH, DE, FR, GB, IT, LU, LI, NL, SE..

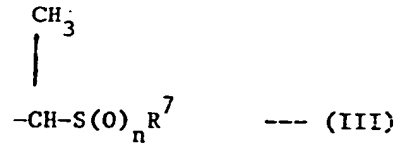
1. A compound having the formula:



(I)

wherein the broken line at the 22-23 position represents an optional double bond and wherein either R^1 is H and the double bond is present or is OH and the double bond is absent;
R^2 is a group of the formula: -CH=CH-R^6; and
R^3 is H or CH_3;
wherein R^6 is H or phenyl or substituted phenyl wherein said substituent is fluorine, chlorine, C_1-C_4 alkyl, C_1-C_4 alkoxy, C_1-C_4 alkylthio, hydroxy(C_1-C_4)alkyl, cyano, aminosulphonyl, C_2-C_6 alkanoyl, C_2-C_6 alkoxycarbonyl, nitro, trifluoromethyl, trifluoromethoxy, amino or mono or di-C_1-C_4 alkylamino.

2. A compound of the formula (I) wherein R^1 and R^3 are as defined in Claim 1 and R^2 is a group of the formula:-



--- (III)

wherein R^7 is C_1-C_4 alkyl, and n is 1 or 2.

3. A compound as claimed in Claim 1 wherein R^2 is -CH=CH_2.

4. A compound as claimed in Claim 1 wherein R^2 is -CH=CH-R^6 and R^6 is 4-trifluoromethoxyphenyl.

5. A compound as claimed in Claim 3 or Claim 4 wherein R^3 is hydrogen, the 22,23 double bond is present and R^1 is hydrogen.

6. A composition for the treatment and prevention of parasitic infections in humans and animals, including ectoparasiticidal, insecticidal, acaricidal and anthelmintic compositions, which comprises a compound of the formula (I) as claimed in any one of Claims 1 to 5 together with an inert diluent or carrier.

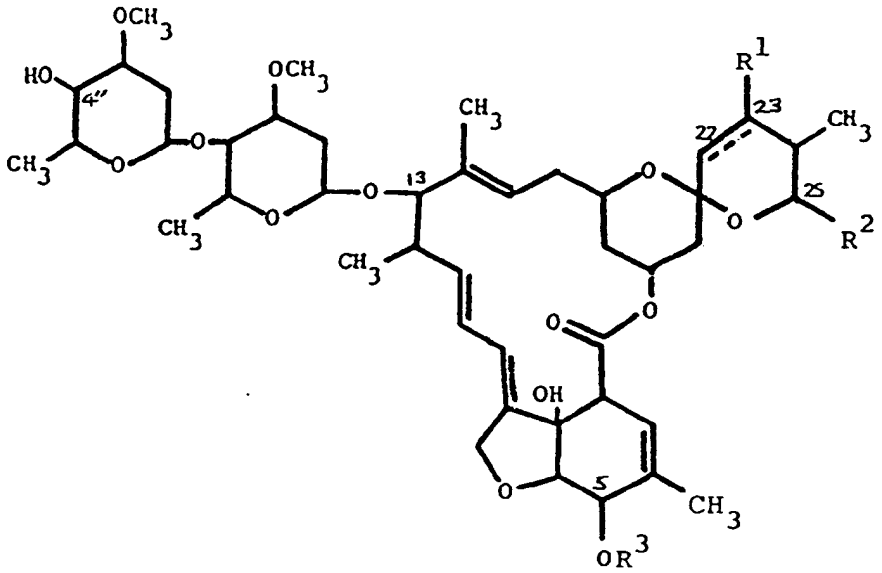
7. A composition as claimed in Claim 6 in the form of an oral, injectable or pour-on formulation.

8. A composition as claimed in Claim 6 in the form of an animal feedstuff or in the form of a premix or supplement for addition to animal feed.

9. A compound of the formula I as claimed in any one of Claims 1 to 5 or a composition thereof as claimed in Claims 6 to 8 for use in the treatment or prevention of parasitic infections in humans and animals.

Claims for the following Contracting States: ES, GR

1. A process for preparing a compound having the formula:



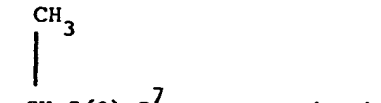
(I)

wherein the broken line at the 22-23 position represents an optional double bond and wherein either R^1 is H and the double bond is present or is OH and the double bond is absent;
R^2 is a group of the formula: -CH=CH-R^6; and
R^3 is H or CH_3;
wherein R^6 is H or phenyl or substituted phenyl wherein said substituent is fluorine, chlorine, C_1-C_4 alkyl, C_1-C_4 alkoxy, C_1-C_4 alkylthio, hydroxy(C_1-C_4)alkyl, cyano, aminosulphonyl, C_2-C_6 alkanoyl, C_2-C_6 alkoxycarbonyl, nitro, trifluoromethyl, trifluoromethoxy, amino or mono or di-C_1-C_4 alkylamino characterised by the following steps:

a) For the preparation of compounds of the formula (I) wherein
R^2 is -CH=CH_2, oxidation of the corresponding C-25 alkylthioalkyl compound of formula (I) wherein R^2 is:

---(II)

Wherein R^7 is C_1-C_4 alkyl to give the corresponding sulphoxide or sulphone wherein R^2 is:



---(III)

wherein n is 1 or 2, followed in the case of the sulphoxides (n=1) by a thermal elimination or, in the case of the sulphones (n=2), by a base catalysed elimination reaction.

(b) For the preparation of compounds of the formula (I) wherein R^2 is -CH=CHR^6 and R^6 is substituted or unsubstituted phenyl reaction of the corresponding compound of formula (I) where R^2 is:

$$-CH = CH_2 \quad (IV)$$

with a compound of the formula R^9-L wherein R^9 is as defined for R^6 and L is a leaving group.

2. A process as claimed in Claim 1 part (a) which comprises oxidising a 25-(1-methylthioethyl)-avermectin with about 1 equivalent of meta-chloroperbenzoic acid and subjecting the resulting 25-(1-methylsulphinylethyl)-avermectin to a thermal elimination reaction to yield the compound of formula (I) wherein R^2 is -CH=CH$_2$.

3. A process as claimed in Claim 1 part (b) which comprises reacting a 25-ethenyl-avermectin with an aryl iodide in the presence of palladium acetate and a tertiary amine to yield the compound of formula (I) wherein R^2 is -CH=CH-R^6 and R^6 is phenyl or substituted phenyl.

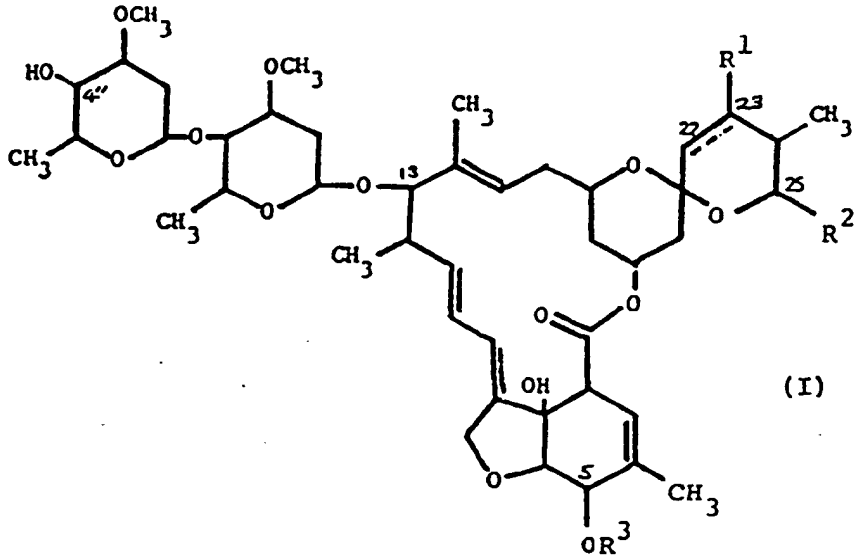
4. A process as claimed in Claim 3 wherein the aryl iodide is 4-trifluoromethoxy-iodobenzene to yield the compound of formula (I) wherein R^2 is 4-trifluoromethoxyphenylethenyl.

5. A process as claimed in any of the Claims 1 to 4 wherein R^3 is hydrogen, R^1 is hydrogen and the 22,23 double bond is present.

Patentansprüche

Patentansprüche für folgende Vertragsstaaten: AT, BE, CH, DE, FR, GB, IT, LU, LI, NL, SE..

1. Verbindung mit der Formel:

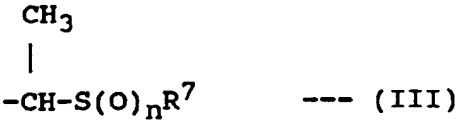


(I)

wobei die durchbrochene Linie an Position 22-23 eine fakultative Doppelbindung bedeutet und wobei entweder R^1 H ist und die Doppelbindung vorhanden ist oder OH bedeutet und keine Doppelbindung vorhanden ist;
R^2 eine Gruppe der Formel -CH=CH-R^6 ist und
R^3 H oder CH_3 ist,
wobei R^6 H oder ein Phenylrest oder ein substituierter Phenylrest ist, worin die Substituenten Fluor, Chlor, C_1-

C_4-Alkyl-, C_1-C_4-Alkoxy-, C_1-C_4-Alkylthio-, Hydroxy(C_1-C_4)alkyl-, Cyano-, Aminosulfonyl-, C_2-C_6-Alkanoyl-, C_2-C_6-Alkoxycarbonyl-, Nitro-, Trifluormethyl-, Trifluormethoxy-, Amino- oder mono- oder di-C_1-C_4-Alkylamino-Gruppen sind.

2. Verbindung der Formel (I), worin R^1 und R^3 wie in Anspruch 1 definiert sind und R^2 eine Gruppe der Formel:



$$-CH(CH_3)-S(O)_nR^7 \qquad \text{--- (III)}$$

ist, worin R^7 ein C_1-C_4-Alkylrest ist und n 1 oder 2 ist.

3. Verbindung nach Anspruch 1, worin R^2 $-CH=CH_2$ ist.

4. Verbindung nach Anspruch 1, worin R^2 $-CH=CH-R^6$ ist und R^6 ein 4-Trifluormethoxyphenylrest ist.

5. Verbindung nach Anspruch 3 oder Anspruch 4, worin R^3 Wasserstoff ist, die Doppelbindung an Position 22, 23 vorhanden ist und R^1 Wasserstoff ist.

6. Zusammensetzung zur Behandlung und Verhütung parasitischer Infektionen bei Menschen und Tieren, einschließend eine ektoparasitizide, insektizide, akarizide und anthelmintische Zusammensetzung, die eine Verbindung der Formel (I) nach einem der Ansprüche 1 bis 5, zusammen mit einem inerten Verdünnungsmittel oder Träger umfaßt.

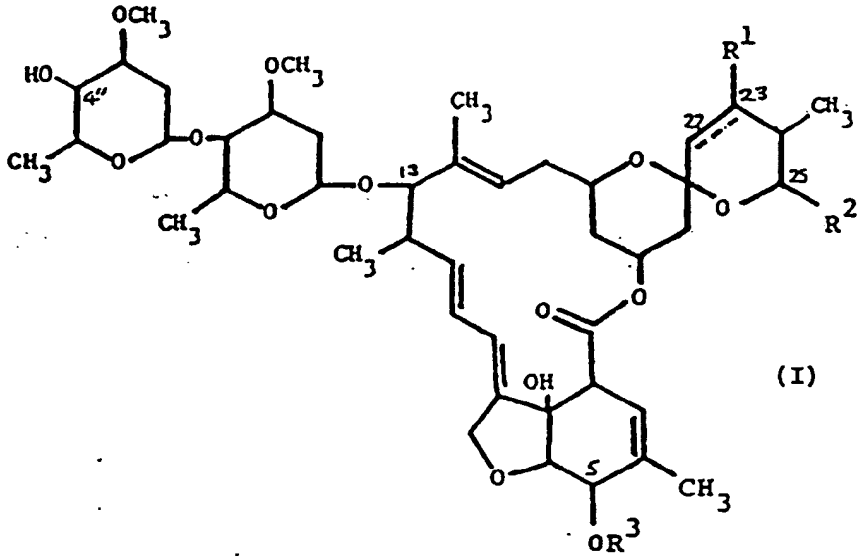
7. Zusammensetzung nach Anspruch 6 in Form eines oralen, injizierbaren oder aufgießbaren Präparates.

8. Zusammensetzung nach Anspruch 6 in Form eines Tierfutters oder in Form einer Vormischung oder Ergänzung zur Zugabe zu Tierfutter.

9. Verbindung der Formel I nach einem der Ansprüche 1 bis 5 oder Zusammensetzung nach einem der Ansprüche 6 bis 8 zur Verwendung zur Behandlung oder Verhütung parasitischer Infektionen bei Menschen und Tieren.

Patentansprüche für folgende Vertragsstaaten: ES, GR

1. Verfahren zur Herstellung einer Verbindung der Formel:



(I)

wobei die durchbrochene Linie an Position 22-23 eine fakultative Doppelbindung bedeutet und wobei entweder R^1 H ist und die Doppelbindung vorhanden ist oder OH bedeutet und keine Doppelbindung vorhanden ist;
R^2 eine Gruppe der Formel $-CH=CH-R^6$ ist und
R^3 H oder CH_3 ist,
wobei R^6 H oder ein Phenylrest oder ein substituierter Phenylrest ist, worin die Substituenten Fluor, Chlor, C_1-

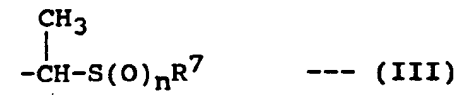
C_4-Alkyl-, C_1-C_4-Alkoxy-, C_1-C_4-Alkylthio-, Hydroxy(C_1-C_4)alkyl-, Cyano-, Aminosulfonyl-, C_2-C_6-Alkanoyl-, C_2-C_6-Alkoxycarbonyl-, Nitro-, Trifluormethyl-, Trifluormethoxy-, Amino- oder mono- oder di-C_1-C_4-Alkylamino-Gruppen sind, gekennzeichnet durch die folgenden Schritte:

a) für die Herstellung der Verbindungen der Formel (I), worin

R^2 -CH=CH_2 ist, Oxidation der entsprechenden C-25-Alkylthioalkyl-Verbindung der Formel (I), worin R^2

--- (II)

ist, worin R^7 ein C_1-C_4-Alkylrest ist, was das entsprechende Sulfoxid oder Sulfon ergibt, worin R^2



--- (III)

ergibt, worin n 1 oder 2 ist und anschließend im Fall der Sulfoxide (n = 1) eine thermische Eliminierung oder im Fall des Sulfons (n = 2) eine Basen-katalysierte Eliminierungsreaktion;

(b) für die Herstellung der Verbindungen der Formel (I), worin

R^2 -CH=CHR^6 ist und R^6 ein substituierter oder unsubstituierter Phenylrest ist, Reaktion der entsprechenden Verbindung der Formel (I), worin R^2

-CH = CH_2 (IV)

ist, mit einer Verbindung der Formel R^9-L, worin R^9 wie R^6 definiert ist und L eine Abgangsgruppe ist.

2. Verfahren nach Anspruch 1, Teil (a) umfassend, daß man ein 25-(1-Methylthioethyl)avermectin mit etwa einem Äquivalent meta-Chlorperbenzoesäure oxidiert und das entstehende 25-(1-Methylsulfinylethyl)avermectin einer thermischen Eliminierungsreaktion unterzieht, was die Verbindung der Formel (I) liefert, worin R^2 -CH=CH_2 ist.

3. Verfahren nach Anspruch 1, Teil (b) umfassend, daß man ein 25-Ethenyl-avermectin mit einem Aryljodid in Gegenwart von Palladiumacetat und einem tertiären Amin umsetzt, was die Verbindung der Formel (I) liefert, worin R^2 -CH=CH-R^6 ist und R^6 ein Phenylrest oder substituierter Phenylrest ist.

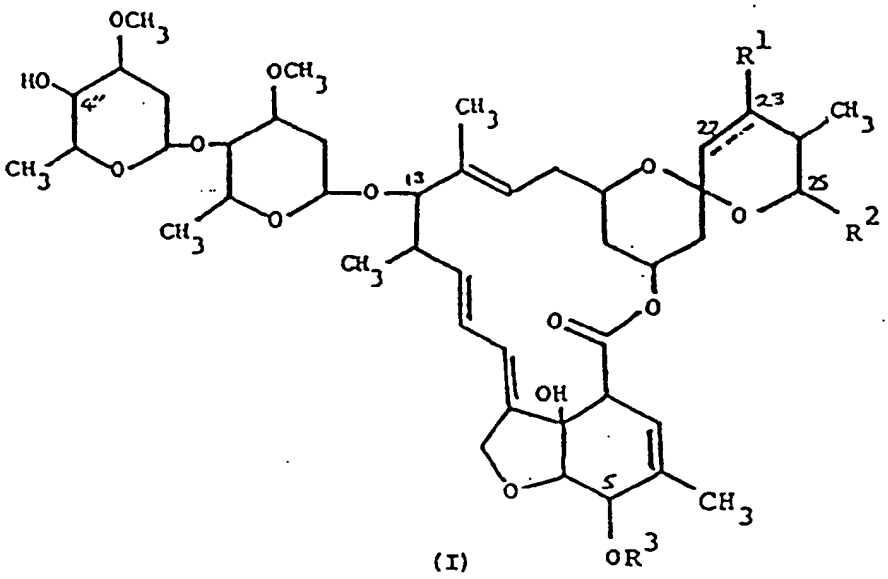
4. Verfahren nach Anspruch 3, worin das Aryljodid 4-Trifluormethoxy-jodbenzol ist, was die Verbindung der Formel (I) liefert, worin R^2 4-Trifluormethoxyphenylethenyl ist.

5. Verfahren nach einem der Ansprüche 1 bis 4, worin R^3 Wasserstoff ist, R^1 Wasserstoff ist und die Doppelbindung an Position 22, 23 vorhanden ist.

Revendications

Revendications pour les Etats contractants suivants: AT, BE, CH, DE, FR, GB, IT, LU, LI, NL, SE..

1. Composé de formule :



(I)

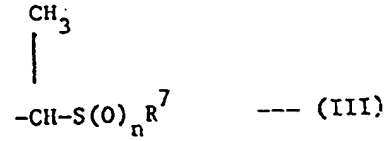
dans laquelle la ligne en pointillés entre les positions 22 et 23 représente une double liaison facultative et dans laquelle soit R^1 représente H et la double liaison est présente, soit R^1 représente OH et la double liaison est absente ;

R^2 est un groupe de formule -CH=CH-R^6 ; et

R^3 représente H ou CH_3 ;

avec R^6 représentant l'hydrogène ou un groupe phényle ou phényl-substitué dans lequel ledit substituant est le fluor, le chlore, un groupe alkyle en C_1-C_4, un groupe alcoxy en C_1-C_4, un groupe (alkyle en C_1-C_4)thio, un groupe hydroxy(alkyle en C_1-C_4), cyano, aminosulfonyle, alcanoyle en C_2-C_6, (alcoxy en C_2-C_6)carbonyle, nitro, trifluorométhyle, trifluorométhoxy, amino ou mono- ou di-(alkyle en C_1-C_4)-amino.

2. Composé de formule (I) dans laquelle R^1 et R^3 sont tels que définis dans la revendication 1, et R^2 est un groupe de formule :



--- (III)

dans laquelle R^7 est un groupe alkyle en C_1-C_4, et n représente 1 ou 2.

3. Composé selon la revendication 1, dans lequel R^2 représente -CH=CH_2.

4. Composé selon la revendication 1, dans lequel R^2 représente -CH=CH-R^6, et R^6 est un groupe 4-trifluorométhoxyphényle.

5. Composé selon la revendication 3 ou 4, dans lequel R^3 représente l'hydrogène, la double liaison en 22,23 est présente, et R^1 représente l'hydrogène.

6. Composition pour le traitement et la prévention des infections parasitaires chez l'homme et l'animal, y compris les compositions ectoparasiticides, insecticides, acaricides et anthelmintiques, qui renferme un composé de formule (I) selon l'une quelconque des revendications 1 à 5 avec un diluant ou un véhicule inerte.

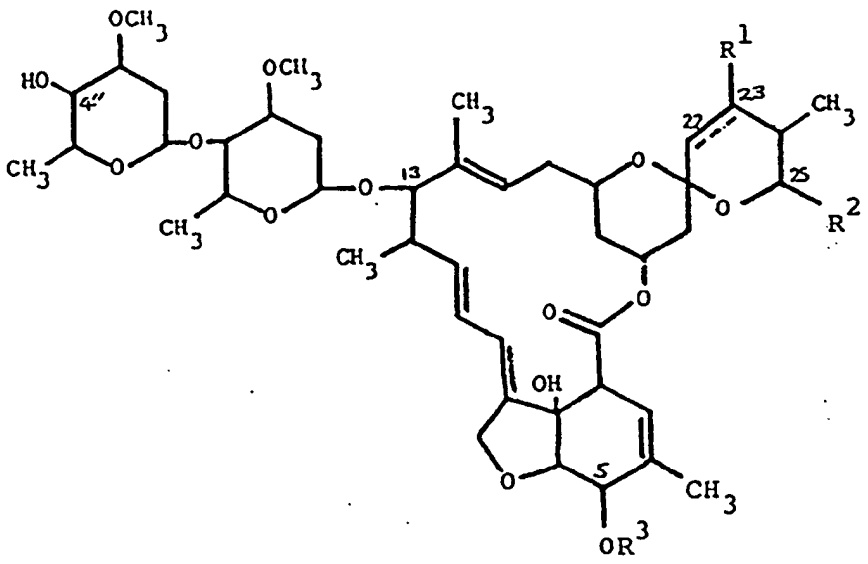
7. Composition selon la revendication 6, en une formulation orale, injectable ou à verser.

8. Composition selon la revendication 6, sous la forme d'un aliment pour animaux ou sous la forme d'un pré-mélange ou d'un supplément à l'alimentation animale.

9. Composé de formule (I) selon l'une quelconque des revendications 1 à 5, ou composition d'un tel composé selon les revendications 6 à 8 pour l'utilisation dans le traitement ou la prévention des infections parasitaires chez l'homme et l'animal.

Revendications pour les Etats contractants suivants: ES, GR.

1. Procédé de préparation d'un composé de formule :



(I)

dans laquelle la ligne en pointillés entre les positions 22 et 23 représente une double liaison facultative et dans laquelle soit R^1 représente H et la double liaison est présente, soit R^1 représente OH et la double liaison est absente ;

R^2 est un groupe de formule -CH=CH-R^6 ; et

R^3 représente H ou CH_3 ;

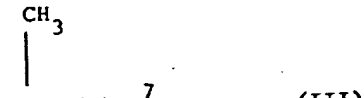
avec R^6 représentant l'hydrogène ou un groupe phényle ou phényl-substitué dans lequel ledit substituant est le fluor, le chlore, un groupe alkyle en C_1-C_4, un groupe alcoxy en C_1-C_4, un groupe (alkyle en C_1-C_4)thio, un groupe hydroxy(alkyle en C_1-C_4), cyano, aminosulfonyle, alcanoyle en C_2-C_6, (alcoxy en C_2-C_6)carbonyle, nitro, trifluorométhyle, trifluorométhoxy, amino ou mono- ou di-(alkyle en C_1-C_4)-amino, caractérisé par les étapes suivantes :

a) pour la préparation des composés de formule (I) dans lesquels R^2 représente -CH=CH_2, l'oxydation sur le C-25 du composé allylthioalkyle de formule (I) correspondant dans laquelle R^2 représente :

---(II)

où R^7 est un groupe alkyle en C_1-C_4

pour donner le sulfoxyde ou la sulfone correspondant dans lequel R^2 représente :



---(III)

où n représente 1 ou 2, suivi dans le cas des sulfoxydes (n=1) par une élimination thermique ou, dans le cas des sulfones (n=2), par une réaction d'élimination catalysée à l'aide d'une base ;

b) pour la préparation des composés de formule (I) dans laquelle R^2 représente -CH=CHR^6 et R^6 représente un groupe phényl-substitué ou non substitué, la réaction du composé correspondant de formule (I) dans laquelle R^2 représente :

-CH = CH_2 (IV)

avec un composé de formule R^9-L où R^9 est tel que défini pour R^6 et L est un groupe labile.

2. Procédé selon la revendication 1 partie (a) qui consiste à oxyder une 25-(1-méthylthioéthyl)-avermectine avec environ 1 équivalent d'acide méta-chloroperbenzoïque, et à soumettre la 25-(1-méthylsulfinyléthyl)-avermectine à une réaction d'élimination thermique pour donner le composé de formule (I) dans laquelle R^2 représente $-CH=CH_2$.

3. Procédé selon la revendication 1 partie (b) qui consiste à faire réagir une 25-éthényl-avermectine avec un iodure d'aryle en présence d'acétate de palladium et d'une amine tertiaire pour donner le composé de formule (I) dans laquelle R^2 représente $-CH=CH-R^6$ et R^6 est un groupe phényle ou phényl-substitué.

4. Procédé selon la revendication 3 dans lequel l'iodure d'aryle est le 4-trifluorométhoxyiodobenzène pour donner le composé de formule (I) dans laquelle R^2 est un groupe 4-trifluorométhoxyphényléthényle.

5. Procédé selon l'une quelconque des revendications 1 à 4 dans laquelle R^3 représente l'hydrogène, R^1 représente l'hydrogène et la double liaison en 22,23 est présente.